Variability in Hand Contamination Based on Serial Measurements: Implications for Assessment of Hand-Cleansing Behavior and Disease Risk

Pavani K. Ram,* Iqbal Jahid, Amal K. Halder, Benjamin Nygren, M. Sirajul Islam, Stewart P. Granger,
John W. Molyneaux, and Stephen P. Luby

*University at Buffalo, Buffalo, New York; International Centre for Diarrhoeal Disease Research (ICDDR,B), Dhaka, Bangladesh;
Emory University, Atlanta, Georgia; Unilever Research and Development, Bebington, Wirral, United Kingdom; 4-Front Research,
Capenhurst, Cheshire, United Kingdom; Millennium Challenge Corporation, Washington, DC*

Abstract. Measuring hand contamination at critical times, such as eating, can be challenging. We examined whether hand contamination measured at random, such as on arrival (initial), predicts contamination at critical times. Mothers of young children in Bangladesh rinsed both hands in 200 mL of ringer's solution. We compared results of serial samples with respect to fecal coliform counts. Among 39 mothers, the geometric mean of fecal coliforms was 307 colony-forming units (cfu)/100 mL at initial collection and 3,001 cfu/100 mL during critical times ($P = 0.0006$). There was no correlation between initial and critical time fecal coliform counts ($R = 0.13$, $P = 0.43$). The mean difference between initial and critical time counts was 3.5 (standard deviation = 1.4) on the log base-10 scale. Contamination of the same subjects' hands varied substantially within a few hours. Because hand contamination measured at random cannot reliably predict hand contamination at times of potential pathogen transmission, single random hand rinses are not valid proxy measures for handwashing behavior.

INTRODUCTION

Handwashing behavior is difficult to measure. A number of self-reported and objective measures have been used. Self-report is not valid, because individuals typically report handwashing more frequently than is seen on observation.[1–3] The direct measure of structured observation, which requires a highly trained observer positioning herself in a subject's home environment for a number of hours, can be time-consuming, expensive, and invasive to the privacy of the subject and her family. Moreover, our recent work has shown that a large proportion of individuals alter their handwashing with soap when they are observed compared with when they are not observed (Ram PK and others, unpublished data). Proxy measures may be more objective than self-report, and they include recording the presence of tools such as soap that facilitate handwashing, observation of hand cleanliness, and measuring hand contamination.[4,5] Hand-contamination measurement seems promising as a proxy method for measuring handwashing behavior, because persons specifically instructed to wash hands with a cleansing agent have been shown to have substantially fewer fecal bacteria contaminating their hands compared with people who have not washed hands with a cleansing agent.[6–8] Some studies have shown a correlation between reduced hand contamination and reduced diarrhea risk.[9–11] However, measured hand contamination can vary based on a number of factors, including the type of sampling and the microbial quantitation methods used,[10] the subject's skin characteristics,[12] humidity,[8] and hygiene behaviors temporally related to the hand-contamination measurement.[6]

Presumably, the dose of pathogens on hands at critical events impacts the degree to which pathogens are actually transmitted, at those times, from hands to other hands, mouths, or food or water vehicles. Detection of those critical events necessitates structured observation. If hand contamination measured at random, at a convenient time for

study personnel, could predict hand contamination at times critical for pathogen transmission, prolonged observation by a human observer could be shortened or avoided, thereby reducing personnel requirements and study costs and increasing the efficiency of data collection. In rural Bangladesh, we examined whether hand contamination, based on convenience sampling of hands, can predict hand contamination at critical events. We also assessed the rate of recontamination of hands after thorough handwashing with soap.

Our study questions were:

(1) Does hand contamination measured at convenient times predict hand contamination at times critical to pathogen transmission?
(2) Does hand contamination measured at one critical time predict hand contamination at another critical time?
(3) What is the rate of recontamination among study subjects 2 hours after thorough handwashing with soap?

MATERIALS AND METHODS

This investigation was nested within a larger study of various methods to measure handwashing behavior performed in six rural villages in Bangladesh. The larger study consisted of 100 participants, all of whom were the primary caregivers of children < 2 years old living in Brahmanbaria or Sirajganj, two districts that lie approximately 2–3 hours outside of the Bangladeshi capital, Dhaka. In rural Bangladesh, households are clustered. In every study village, we visited each cluster with at least one child less than 2 years old. If the cluster had only one child less than 2 years old, we enrolled that child's primary caregiver. If the cluster had multiple children less than 2 years old, we used systematic random sampling to select one caregiver for participation. The fieldworker asked all primary caregivers of a child < 2 years old in the cluster to stand in front of their household. Starting at the entrance of the cluster, the fieldworker worked from her right in a circular fashion to count off the primary caregivers from 1 to 5. The primary caregiver counted as 5 was asked to take part in the study. For example, if there were three eligible caregivers in the cluster, the second caregiver from the right would have been counted

* Address correspondence to Pavani K. Ram, University at Buffalo, Room 270, Farber Hall, 3435 Main Street, Buffalo, NY 14221. E-mail: pkram@buffalo.edu

as 2 and again as 5 and thus, would have been requested to participate.

For the purposes of this investigation, hand-rinse samples were collected from a total of 55 participants (Table 1). Among these, 25 participants were randomly assigned to a 5-hour structured observation, and 30 participants were assigned to a 90-minute structured observation. From all, the field worker collected a hand-rinse sample immediately on arrival. This sample will hereafter be referred to as the initial sample, because it was not timed to correspond to any specific critical event. In the 90-minute observation group, field workers collected one hand-rinse sample timed to correspond to a critical event (critical time 1) in addition to the initial sample. In the 5-hour observation group, field workers collected two hand-rinse samples at critical events in addition to the initial sample (critical times 1 and 2). Critical events of interest included the following: before eating, before feeding a child, before drinking, and before storing water.

In households in the 90-minute observation group, the participant was also requested to wash her hands thoroughly with soap after the conclusion of the structured observation; no specific instructions were given regarding how hands should be dried. The interviewer returned to the household 2 hours later to collect a final hand-rinse sample to estimate the rate of recontamination. This sample will hereafter be referred to as the recontamination sample.

Two-liter Whirl-pak bags were pre-filled with 200 mL of sterile Ringer's solution each, and the requisite number of bags was provided to field workers before data collection began each day. Before the collection of a hand-rinse sample, the field worker cleansed both of her hands with an alcohol-based waterless hand sanitizer and air-dried her hands. She then opened a Whirl-pak bag and requested the participant to insert one fist into the bag (Figure 1). The field worker held the bag from the outside, assisting the participant to ensure that her entire hand came into contact with the Ringer's solution. The participant was asked to rub her fingers and palm against each other for a total of 10 times and rub her thumb against the nails on each of the other four fingers. Both hands were rinsed sequentially in the same bag in similar fashion. After both hands were rinsed in the bag, the field worker closed the bag and placed it immediately into a cold box with ice packs, which maintained the box at ≤ 10°C. The hand rinse samples were transported to the Environmental Microbiology Laboratory of ICDDR, B and processed for microbiological analysis within 24 h of collection.

The hand-rinse sample was processed using standard membrane filtration techniques to quantify the number of colony forming units (cfu) of fecal coliforms and *Escherichia coli*.

FIGURE 1. Collection of hand-rinse samples from a participant in Bangladesh in 2007.

Ten-milliliter samples were initially filtered through 0.22-μm Millipore (Billerica, MA) membrane filters and plated on to modified fecal coliform (mFC) and modified thermotolerant *E. coli* (mTEC) agar media. After the 10-mL samples were plated, the samples were stored at 4°C to prevent multiplication. If the number of colony-forming units were too numerous to count, then 0.1 mL or 100 μL of the samples or 10-fold dilutions of the samples were plated on mFC and mTEC agar media on the next day. The dilution technique used has been described previously.[13] Fifteen percent of the samples were initially read as too numerous to count (TNTC) and required testing following 10-fold dilution. All mFC plates were incubated at 44°C for 18–24 hours after plating, and on the next day. All mTEC media were first incubated at 37°C for 2 hours; then, the plates were transferred at 44°C and incubated for 18–24 hours, and the blue colonies from mTEC agar were counted as thermotolerant *E. coli*.

We estimated that we would be able to collect hand-rinse samples from a total of 50 participants based on logistic and budgetary limits.

Study enrollment was entirely voluntary, and all participants provided written informed consent. The medium used for hand-rinse collection is commonly used in microbiologic studies performed on human subjects. The study protocol was reviewed and approved by the Research and Ethical Review Committees of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), and the Social and Behavioral Sciences Institutional Review Board of the University at Buffalo.

DATA ANALYSIS

We calculated the levels of hand contamination in the hand rinse according to log (base 10)-transformed counts of colony forming units per 100 mL of fecal coliforms and *E. coli*. Because of the wide range in the raw counts of colony forming units per 100 mL of fecal coliforms or *E. coli* at each sampling time, we report geometric means of the raw counts. To calculate geometric means, we assigned an arbitrary value of

TABLE 1
Timing of hand-rinse sampling among caregivers of children < 2 years old in rural Bangladesh in 2007

	5-hour structured observation group	90-minute structured observation group
N	25	30
Hand-rinse sampling time		
Initial	✓	✓
Critical time 1*	✓	✓
Critical time 2*	✓	
Recontamination		✓

*Critical events of interest were before eating, before feeding a child, before drinking, and before storing water.

0.5 cfu/100 mL for hand-rinse samples with no detectable colony-forming units. We performed paired *t* tests to assess for significant differences between levels of contamination at two sampling times. To address the question of whether contamination measured during random hand-rinse samples predicts contamination at critical times, we performed Spearman's rank correlation to estimate the correlation between the degree of contamination in initial hand-rinse samples and the degree of contamination in hand-rinse samples collected at critical time 1 or 2. To provide a unit of measure of the difference in contamination between two sampling times, we calculated the difference in counts of fecal coliforms or *E. coli* at initial and critical time 1 or 2, calculated the logarithm of the absolute value of the difference using base 10, and took the mean of the log-transformed differences.

We calculated the frequency of recontamination, which was defined as the presence of fecal coliforms or *E. coli* (> 0 cfu/100 mL) in the hand-rinse sample that was collected 2 hours after a thorough handwashing with soap. In addition, we calculated the geometric mean of the number of colony forming units per 100 mL of fecal coliforms and *E. coli* in the recontamination sample.

RESULTS

For this investigation, we enrolled a total of 55 participants, 25 in the 5-hour observation group and 30 in the 90-minute observation group. All participants enrolled were the mothers of children < 2 years old. The mean age of participants was 25.7 years (standard deviation [SD] = 5.6, range = 16–39). Participants reported a mean of 5.5 years of education (SD = 3.7, range = 0–12) for themselves and 4.6 years of education for the heads of household (SD = 4.0, range = 0–12).

Initial hand-rinse samples were taken from all 55 participants. For 5 of 55 participants, the observations were interrupted after the initial hand-rinse sampling was completed because of the sudden onset of intense monsoon rains that resulted in flooding of households and villages on the day of data collection. A sixth participant, who was in the 5-hour observation group, provided the initial hand-rinse sample but was not available for either critical time 1 or 2 sampling. In 10 (20%) households, no critical event was observed; all of these households were in the 90-minute observation group. Since the primary objective of this study was to examine the relationship between hand contamination at convenient times and hand contamination at specific critical events, we excluded these 10 participants from further analysis. For 3 (13%) participants, only one critical event was observed and, thus, no critical time 2 sample could be taken; these 3 samples were excluded from analysis of critical time 2 data. Thus, hand rinse data were available from 39 participants for critical time 1 and from 21 participants for critical time 2 (Table 2).

All structured observations began mid-day, with the Initial sample collected between 10:55 am and 3:50 pm. Counts of fecal coliforms and *E. coli* were greatest in the critical time 1 sampling, compared to Initial and critical time 2 sampling (Table 2). Hand rinses from 39 participants were taken for critical time 1 at the following opportunities for handwashing: before food preparation (72%), before feeding a child (23%), before eating (3%), and before drinking water (3%) (Table 3).

TABLE 2
Geometric means (colony-forming units per 100 mL) of fecal coliforms and *E. coli* in hand-rinse samples from participating caregivers of children < 2 years old in rural communities in Bangladesh in 2007

Variable	N	Geometric mean of raw counts
Initial (fecal coliforms)	39	307
Initial (*E. coli*)	39	19
Time 1 (fecal coliforms)	39	3001
Time 1 (*E. coli*)	39	46
Time 2 (fecal coliforms)	21	512
Time 2 (*E. coli*)	21	34
Recontamination assessment (fecal coliforms)	25	494
Recontamination assessment (*E. coli*)	25	17

The geometric mean count of fecal coliforms at initial collection was 307 cfu/100 mL compared with 3,001 cfu/100 mL at critical time 1 ($P = 0.0006$). The geometric mean count of *E. coli* at initial collection was 19 cfu/100 mL compared with 46 cfu/100 mL at critical time 1 ($P = 0.15$). There was no correlation between the log-transformed count of fecal coliforms (Table 4) and *E. coli* (Table 5) detected in initial and critical time 1 samples. The mean absolute difference of fecal coliforms between initial and critical time 1 sampling was 3.5 (SD = 1.4) on the logarithmic (base 10) scale. The mean absolute difference of *E. coli* between initial and critical time 1 sampling was 2.1 (SD = 0.8) on the logarithmic (base 10) scale. The Bland–Altman plot shows that the absolute difference in fecal coliform counts was strongly correlated with the mean of the fecal coliform counts from initial and critical time 1 sampling ($R = 0.96, P < 0.0001$) (Figure 2). The absolute difference was strongly correlated with the mean of the *E. coli* counts from initial and critical time 1 sampling ($R = 0.98, P < 0.0001$).

Hand rinses from 21 participants, all in the 5-hour observation group, were taken for critical time 2 during at following opportunities for handwashing: before food preparation (47%), before feeding a child (29%), before eating (19%), and before handling water for storage (5%). The geometric mean count of fecal coliforms in the initial sample was 329 cfu/100 mL compared with 512 cfu/100 mL at critical time 2 ($P = 0.63$). The geometric mean count of *E. coli* taken in the initial sample was 24 cfu/100 mL compared with 34 cfu/100 mL at critical time 2 ($P = 0.67$). There was no correlation between the log-transformed results of fecal coliforms (Table 4) or *E. coli* (Table 5) detected in initial and critical time 2 samples. The mean of the absolute difference between initial and critical time 2 sampling was 2.9 (SD = 1.5) for fecal coliform counts and 2.0 (SD = 0.7) for *E. coli* counts on the logarithmic (base 10) scale. The absolute difference was strongly correlated with the mean for both the fecal coliform counts ($R = 0.92$,

TABLE 3
Frequencies of critical events at which hand-rinse samples were collected among participating caregivers of children < 2 years old in rural communities in Bangladesh in 2007

Critical events	Critical time 1	Critical time 2
Food preparation	72%	48%
Feeding a child	23%	29%
Eating	3%	19%
Drinking water	3%	0%
Handling water for storage	0%	5%

TABLE 4
Correlations and differences between fecal coliform counts detected in hand-rinse samples among participating caregivers of children < 2 years old in rural communities in Bangladesh in 2007

	Initial	Critical time 1	Critical time 2	Recontamination
Initial	–	$N = 39$, $R = 0.13$, $P = 0.43$, MAD* = 3.5 (SD = 1.4)	$N = 21$, $R = -0.09$, $P = 0.68$, MAD* = 2.9 (SD = 1.5)	$N = 25$, $R = 0.41$, $P = 0.04$, MAD* = 2.9 (SD = 1.3)
Critical time 1	$N = 39$, $R = 0.13$, $P = 0.43$, MAD* = 3.5 (SD = 1.5)	–	$N = 21$, $R = 0.44$, $P = 0.05$, MAD* = 3.4 (SD = 1.5)	–
Critical time 2	$N = 21$, $R = -0.09$, $P = 0.68$, MAD* = 2.9 (SD = 1.5)	$N = 21$, $R = 0.44$, $P = 0.05$, MAD* = 3.4 (SD = 1.5)	–	–
Recontamination	$N = 25$, $R = 0.41$, $P = 0.04$, MAD* = 2.9 (SD = 1.3)	–	–	–

*MAD refers to the mean of the log (base 10)-transformed absolute differences between the two sets of counts of fecal coliforms.

$P < 0.0001$) and the *E. coli* counts ($R = 0.93$, $P < 0.0001$) from initial and critical time 2 sampling.

For 21 participants from the 5-hour structured observation group, we had the opportunity to compare results of hand rinses collected at critical times 1 and 2. The frequencies of the specific opportunities for hand-rinse sampling among the 5-hour structured observation group are detailed in Table 3. The geometric mean count of fecal coliforms at critical time 1 was 1,989 cfu/100 mL compared with 512 cfu/100 mL at critical time 2 ($P = 0.06$). The geometric mean count of *E. coli* at critical time 1 was 60 cfu/100 mL compared with 34 cfu/100 mL at critical time 2 ($P = 0.31$). The correlation in hand contamination at critical time 1 and 2 was significant for fecal coliforms ($R = 0.44$, $P = 0.05$) and *E. coli* ($R = 0.47$, $P = 0.03$). The mean of the absolute difference in fecal coliforms between critical times 1 and 2 sampling was 3.4 (SD = 1.5) on the logarithmic (base 10) scale. The mean absolute difference in *E. coli* counts between critical times 1 and 2 sampling was 1.8 (SD = 1.1) on the logarithmic (base 10) scale. The absolute difference was strongly correlated with the mean for both the fecal coliform counts ($R = 0.98$, $P < 0.0001$) and the *E. coli* counts ($R = 0.92$, $P < 0.0001$).

Under the supervision of our fieldworkers, 25 participants performed thorough handwashing with soap. At approximately 2 hours after the thorough handwashing, all participants had detectable fecal coliforms, with a geometric mean count of 494 cfu/100 mL. The geometric mean count of *E. coli* was 17 cfu/100 mL in the recontamination sample. There was no detectable *E. coli* on the hands of five (20%) mothers, with quartile cut-offs at 15 (25%), 26 (50%), and 120 (75%) cfu/100 mL. The highest count of *E. coli* was 3,580 cfu/100 mL. We found correlations between the results of the initial sample and the recontamination sample for both fecal coliforms ($R = 0.41$, $P = 0.04$) and *E. coli* ($R = 0.44$, $P = 0.03$). The absolute difference was strongly correlated with the mean for both

the fecal coliform counts ($R = 0.98$, $P < 0.0001$) and the *E. coli* counts ($R = 0.97$, $P < 0.0001$).

DISCUSSION

This investigation confirmed that measured microbiological hand contamination varies substantially from one sampling time to another. Hand contamination conveniently measured at an unannounced visit is not well-correlated with hand contamination measured at critical times when pathogens may be transmitted from hands to other persons or vehicles, such as food or water, pointing to the high variability and lack of reliability of hand microbiology testing. Thus, hand contamination measured at a convenient time, such as immediately on arrival of the investigator, does not serve as a useful proxy for hand contamination that might be present at a critical event. The Bland–Altman plots confirmed that differences in results of serially collected hand-rinse specimens increase as overall hand contamination increases, indicating that variability may be greatest where hand contamination is greatest. In our study, all participants had recontamination of hands with fecal coliforms, and the majority showed contamination with *E. coli* shortly after a thorough handwashing with soap, indicating that hands become contaminated quickly in an environment that is likely heavily fecally contaminated.[14]

Measurement of hand contamination has been performed to determine exposure to fecal pathogens, assess the individual's overall hand hygiene, and assess the impact of handwashing promotion.[6–9,12,15,16] Hand contamination is attractive as a measure of handwashing behavior, because it seems objective. However, the degree of variability in hand contamination, as well as the rapid recontamination of hands identified in this study call into question the validity of a single hand-rinse sample as a proxy measure for handwashing behavior. Without further studies to assess the relationships between

TABLE 5
Correlations and differences between log-transformed *E. coli* counts detected in hand-rinse samples among participating caregivers of children < 2 years old in rural communities in Bangladesh in 2007

	Initial	Critical time 1	Critical time 2	Recontamination
Initial	–	$N = 39$, $R = -0.22$, $P = 0.18$, MAD* = 2.1 (SD = 0.8)	$N = 21$, $R = 0.02$, $P = 0.93$, MAD* = 2.0 (SD = 0.8)	$N = 25$, $R = 0.44$, $P = 0.03$, MAD* = 1.7 (SD = 1.1)
Critical time 1	$N = 39$, $R = -0.22$, $P = 0.18$, MAD* = 2.1 (SD = 0.8)	–	$N = 21$, $R = 0.47$, $P = 0.03$, MAD* = 1.8 (SD = 1.1)	–
Critical time 2	$N = 21$, $R = 0.02$, $P = 0.93$, MAD* = 2.9 (SD = 0.8)	$N = 21$, $R = 0.47$, $P = 0.03$, MAD* = 1.8 (SD = 1.1)	–	–
Recontamination	$N = 25$, $R = 0.44$, $P = 0.03$, MAD* = 1.7 (SD = 1.1)	–	–	–

*MAD refers to the mean of the log (base 10)-transformed absolute differences between the two sets of counts of *E. coli*.

Mean absolute difference= 3.5 (SD 1.4)
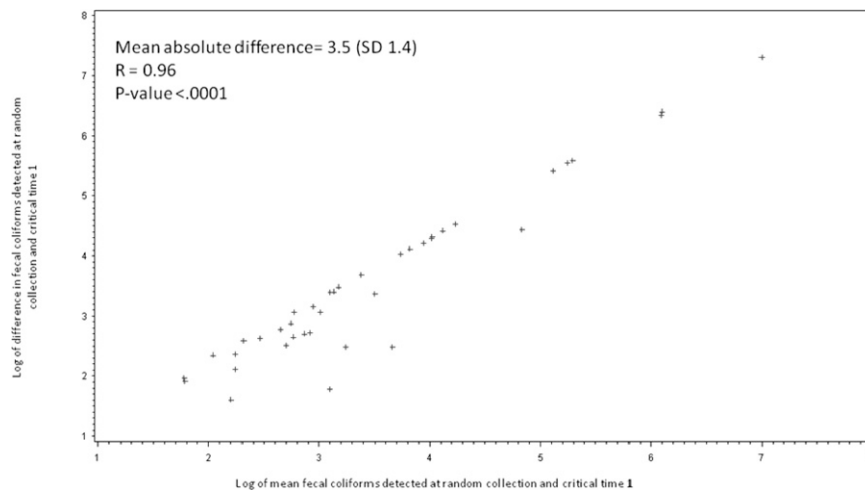R = 0.96
P-value <.0001

FIGURE 2.   Bland–Altman plot of correlation between difference between initial and critical time 1 fecal coliform counts and mean of initial and critical time 1 fecal coliform counts.

hand contamination and disease risk and hand contamination and handwashing behavior, as measured by direct observation techniques, as well as to test strategies to minimize variability in hand-contamination testing, we cannot recommend single hand rinses for measurement of handwashing behavior.

There is little information from previous studies to explain the degree of variability in hand contamination found here. Our finding that differences between two samples increased as the mean colony counts increased suggests that variability increases as overall contamination levels increase. We speculate that variability in hand microbiology could result from any of the following factors: duration since last use of soap or mud for handwashing, duration since last defecation or contact with a child's feces, choice of which hand is used for cleansing oneself after defecation, and overall fecal contamination of the subject's environment. Ambient humidity has been shown to be correlated with fingertip contamination.[8] Among persons who do wash hands with soap, the duration of wash time with soap and the volume of soap used for handwashing may also impact the level of hand contamination detected.[17] Of course, factors related to the sample collection and the microbiological tests themselves, including the inconsistency in rigor and technique of rinsing the respondent's hands during sample collection, the methodology used to count the number of organisms or colonies of organisms, and the consistency in the media and other materials, may be responsible for variability in the levels of hand contamination detected.

The degree of difference between levels of hand contamination in the serial hand-rinse samples in our study was two to three orders of magnitude, even though we found correlations in hand contamination measured initially and in the recontamination sample, and between the two critical times. Are such differences between serial hand-rinse samples meaningful? There is no information from previous hand-hygiene research about whether this degree of difference in the concentration of indicator organisms on hands represents sufficiently greater exposure to pathogens and thus, a higher risk of diarrhea or respiratory infection for the subject herself or the children under her care. Thus, we turn to the available information on household water contamination for corollary evidence of the relevance of varying degrees of contamination for disease risk. Moe and others found that drinking water contaminated with ≥ 1,000

E. coli per 100 mL compared with drinking water with 2–100 *E. coli* per 100 mL, representing one to two orders of magnitude difference, conferred a significantly increased risk of diarrhea in the Philippines.[18] In a more recent study of the biosand filter in the Dominican Republic, Stauber and others found a geometric mean concentration of 19 *E. coli* per 100 mL in control households and 11 *E. coli* per 100 mL in households with the biosand filter; despite this difference in the level of *E. coli* contamination of less than one order of magnitude, the incidence rate ratio for diarrheal disease in the biosand filter households, using the control households as a referent group, was 0.47 (95% confidence interval [CI] = 0.37–0.59).[19] Using households with < 1 *E. coli* per 100 mL in drinking water as the referent group, Brown and others[16] found that two orders of magnitude difference resulted in significantly increased diarrhea risk.[20]

Although these studies of household water quality do suggest that one or two orders of magnitude difference in contamination of a household exposure represent increasing disease risk, we must be judicious in using them to interpret our findings with respect to hand contamination. Hands may serve as direct vehicles of pathogen transmission to the subject herself or those with whom she comes into contact; in this context, just a few organisms of a pathogen that requires only a low infectious dose may serve to cause disease. Alternatively, if just a few organisms of a pathogen that requires a moderate to high infectious dose to establish disease are found on hands, disease risk may be minimal if the pathogen is transmitted directly from hands to the subject herself or others around her. However, hands may also serve as primary vehicles of pathogen transmission to secondary vehicles, such as food or drinking water. In this case, even a few organisms of a low-infectious dose pathogen may be sufficient to establish contamination of the secondary vehicle, thereby increasing the disease risk of all who are exposed to that food or drinking water. We look to the degree of hand contamination to tell us about hand-cleansing behavior and because we assume that the level of contamination detected on hands informs us about the risk of disease for the individual whose hands are tested or for those under her care, such as young children. Given the complexities of pathogen transmission from hands directly or indirectly to susceptible persons, even one order of magnitude

difference in serial measurements of hand contamination may represent substantial difference in the disease risk of the individuals under study.

A practical implication of the lack of correlation in hand contamination at convenient collection and critical event-based collection is that the researcher who wishes to examine hand contamination at critical events must plan for structured observation to detect those events. Structured observation can be time-consuming and inefficient, particularly if the researcher is interested in behavior at specific events, such as feeding a child or cleansing after defecation. The costs of conducting structured observation could be prohibitive for large sample sizes, particularly for meagerly funded research studies or program evaluations being carried out in resource-poor settings.

Similar to our finding of a high rate of hand recontamination, Sobel and others found that, at 1 hour after thorough handwashing, 46% and 23% of street food and beverage vendors, respectively, in Guatemala had measurable fecal coliforms and *E. coli* on hands.[14] In our recontamination assessment, 75% of participants had *E. coli* counts ≥ 15 cfu. We do not have information from prior research on whether this degree of hand contamination is reflective of increased disease risk. Moreover, our limited sample size prevented us from assessing factors associated with recontamination. In the research context, it would be useful to pair hand-contamination measurement with structured observation to understand which activities confer the greatest risk of hand recontamination and to assess which hand-cleansing behaviors confer the greatest protection from hand recontamination. Such data could inform handwashing promotion campaigns regarding behaviors that prevent or contribute to recontamination.

In hand-rinse sampling, we measure indicator organisms (fecal coliforms and *E. coli*) rather than counts of pathogenic organisms. Because of this limitation, we cannot be certain that variability in counts of indicator organisms is reflective of variability in counts of pathogenic organisms. The sample size in this study was small because of logistical and budgetary constraints. Were results of serial hand-rinse samples highly correlated, even a small sample size should have been sufficient to detect such correlations. The small sample size did prevent us from exploring in-depth factors that might contribute to variability. We were in a relatively restricted geographical area of Bangladesh. It is possible that ambient environmental conditions, such as temperature and humidity, may have uniquely affected microbial growth in the hand-rinse samples. It would be worth examining variability in serial hand-rinse samples among participants living in different geographic and environmental conditions. Budgetary constraints prevented us from measuring hand contamination immediately following the supervised thorough handwashing and, thus, we cannot be sure that hands were entirely free of organisms as soon as hands were washed. It is possible that the organisms detected on hands in the recontamination sample were not as a result of recontamination but, rather, just residual contamination since handwashing does not always lead to complete elimination of organisms from hands.[21] Still, the implications for pathogen transmission would be expected to be similar, irrespective of whether hands are residually contaminated or recontaminated.

Hand-contamination measurement can be costly when using standard methods (?US $10 per test in a water microbiology reference laboratory in Bangladesh). Despite the cost, our findings indicate that single measures of microbiological hand contamination may not accurately represent hand contamination of the individual over time and thus, may not be adequate proxies for handwashing behavior. We need to understand the factors contributing to variability of hand-contamination measurement and whether these factors may be modified to improve reliability of this potentially useful technique. Further studies are needed to clarify whether hand contamination measured by single hand rinses is associated with observed handwashing behavior and disease risk before hand contamination can be used as a proxy measure of handwashing practice.

Authors' addresses: Pavani K. Ram, University at Buffalo, Buffalo, NY, E-mail: pkram@buffalo.edu. Iqbal Jahid, Amal K. Halder, M. Sirajul Islam, and Stephen P. Luby, International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Shaheed Tajuddin Ahmed Sharani, Mohakhali, Dhaka, Bangladesh, E-mails: sislam@icddrb.org, Amal_Halder@wvi.org, sislam@icddrb.org, and sluby@icddrb.org. Benjamin Nygren, Rollins School of Public Health, Emory University, Atlanta, GA, E-mail: ghz8@cdc.gov. Stewart P. Granger, Unilever Research and Development Port Sunlight, Bebington, Wirral, United Kingdom, E-mail: stewart.granger@southcentral.nhs.uk. John W. Molyneaux, Water and Sanitation Program, The World Bank Group, Washington, DC, E-mail: jack_molyneaux@usa.net.

REFERENCES

1. Biran A, Rabie T, Schmidt W, Juvekar S, Hirve S, Curtis V, 2008. Comparing the performance of indicators of hand-washing practices in rural Indian households. *Trop Med Int Health 13:* 278–285.

2. Manun'Ebo M, Cousens S, Haggerty P, Kalengaie M, Ashworth A, Kirkwood B, 1997. Measuring hygiene practices: a comparison of questionnaires with direct observations in rural Zaire. *Trop Med Int Health 2:* 1015–1021.

3. Stanton BF, Clemens JD, Aziz KM, Rahman M, 1987. Twenty-four-hour recall, knowledge-attitude-practice questionnaires, and direct observations of sanitary practices: a comparative study. *Bull World Health Organ 65:* 217–222.

4. Luby SP, Halder AK, 2008. Associations among handwashing indicators, wealth, and symptoms of childhood respiratory illness in urban Bangladesh. *Trop Med Int Health 13:* 835-8-44.

5. Halder AK, Tronchet C, Akhter S, Bhuiya A, Johnston R, Luby SP, Observed hand cleanliness and other measures of handwashing behavior in rural Bangladesh. *BMC Public Health 10:* 545.

6. Hoque BA, Mahalanabis D, Alam MJ, Islam MS, 1995. Post-defecation handwashing in Bangladesh: practice and efficiency perspectives. *Public Health 109:* 15–24.

7. Luby SP, Agboatwalla M, Raza A, Sobel J, Mint ED, Baier K, Hoekstra RM, Rahbar MH, Hassan R, Qureshi SM, Gangarosa EJ, 2001. Microbiologic effectiveness of hand washing with soap in an urban squatter settlement, Karachi, Pakistan. *Epidemiol Infect 127:* 237–244.

8. Pinfold JV, 1990. Faecal contamination of water and fingertip-rinses as a method for evaluating the effect of low-cost water supply and sanitation activities on faeco-oral disease transmission. I. A case study in rural north-east Thailand. *Epidemiol Infect 105:* 363–375.

9. Pinfold JV, Horan NJ, 1996. Measuring the effect of a hygiene behaviour intervention by indicators of behaviour and diarrhoeal disease. *Trans R Soc Trop Med Hyg 90:* 366–371.

10. Luby SP, Agboatwalla M, Billhimer W, Hoekstra RM, 2007. Field trial of a low cost method to evaluate hand cleanliness. *Trop Med Int Health 12:* 765–771.

11. Pickering AJ, Davis J, Walters SP, Horak HM, Keymer DP, Mushi D, Strickfaden R, Chynoweth JS, Liu J, Blum A, Rogers K, Boehm AB. Hands, water, and health: fecal contamination in Tanzanian communities with improved, non-networked water supplies. *Environ Sci Technol 44:* 3267–3272.

12. Larson E, Aiello A, Lee LV, Della-Latta P, Gomez-Duarte C, Lin S, 2003. Short- and long-term effects of handwashing with antimicrobial or plain soap in the community. *J Community Health 28:* 139–150.

13. Islam MS, Alam MJ, Khan SI, Huq A, 1994. Faecal pollution of fresh water environments in Bangladesh. *International Journal of Environmental Studies 46:* 161–165.

14. Sobel J, Mahon B, Mendoza CE, Passaro D, Cano F, Baier K, Racioppi F, Hutwagner L, Mintz E, 1998. Reduction of fecal contamination of street-vended beverages in Guatemala by a simple system for water purification and storage, hand-washing, and beverage storage. *Am J Trop Med Hyg 59:* 380–387.

15. Kaltenthaler E, Waterman R, Cross P, 1991. Faecal indicator bacteria on the hands and the effectiveness of hand-washing in Zimbabwe. *J Trop Med Hyg 94:* 358–363.

16. Kaltenthaler EC, Pinfold JV, 1995. Microbiological methods for assessing handwashing practice in hygiene behaviour studies. *J Trop Med Hyg 98:* 101–106.

17. Fuls JL, Rodgers ND, Fischler GE, Howard JM, Patel M, Weidner PL, Duran MH, 2008. Alternative hand contamination technique to compare the activities of antimicrobial and nonantimicrobial soaps under different test conditions. *Appl Environ Microbiol 74:* 3739–3744.

18. Moe CL, Sobsey MD, Samsa GP, Mesolo V, 1991. Bacterial indicators of risk of diarrhoeal disease from drinking-water in the Philippines. *Bull World Health Organ 69:* 305–317.

19. Stauber CE, Ortiz GM, Loomis DP, Sobsey MD, 2009. A randomized controlled trial of the concrete biosand filter and its impact on diarrheal disease in Bonao, Dominican Republic. *Am J Trop Med Hyg 80:* 286–293.

20. Brown JM, Proum S, Sobsey MD, 2008. *Escherichia coli* in household drinking water and diarrheal disease risk: evidence from Cambodia. *Water Sci Technol 58:* 757–763.

21. Pickering AJ, Boehm AB, Mwanjali M, Davis J, 2010. Efficacy of waterless hand hygiene compared with handwashing with soap: a field study in Dar es Salaam, Tanzania. *Am J Trop Med Hyg 82:* 270–278.